An intrinsic thyrotropin-mediated pathway of TNFα production by bone marrow cells

Heuy-Ching Wang, Jolene Dragoo, Qin Zhou, and John R. Klein

From the Department of Basic Sciences, Dental Branch, University of Texas Health Science Center at Houston, Houston, TX 77030

Running Head: TSH MODULATION OF BONE MARROW TNFα PRODUCTION

Total word count: 3,278

Abstract word count: 238

Scientific heading: Hematopoiesis

This work was supported in part by NIH grant DK35566

Corresponding author:

Dr. John R. Klein
University of Texas Health Science Center Houston
Department of Basic Sciences
Dental Branch, Rm 4.133
6516 MD Anderson Blvd
Houston, TX 77030
TEL: 713-500-4369
FAX: 713-500-4500
e-mail: John.R.Klein@uth.tmc.edu

Copyright 2002 American Society of Hematology
Abstract

Recent studies have identified a role for thyroid stimulating hormone (TSH, i.e., thyrotropin) as an inductive signal for tumor necrosis factor-α (TNFα) secretion by bone marrow (BM) cells, though the features of that activational pathway have not been defined. Using intracellular TSH staining and enzyme-linked immunoassay for detection of secreted TSH, we demonstrate that TSH synthesis in BM cells occurs within CD45+ (leukocyte-common antigen) hematopoietic cells, and that the majority of that activity resides in a component of CD11b+ BM cells that are not mature T cells, B cells, or Thy-1+ cells in the BM. Conversely, TSH-responsive BM cells defined by expression of TSH receptor (TSHR) using flow cytometry were selectively associated with a non-erythroid CD11b− lymphocyte precursor population. In vitro culture of magnetic-activated cell sorted CD11b− and CD11b+ cells with titrated amounts of purified TSH resulted in significantly higher levels of TNFα secretion from CD11b− BM cells compared to non-TSH-treated cells, with no appreciable change in TNFα production from CD11b+ cells. These findings are the first to demonstrate TSH production by BM hematopoietic cells, and they demonstrate that TSH may be involved in the regulation of TNFα by CD11b− BM cells. They also indicate that TSH-mediated regulation of TNFα secretion within the BM most likely operates through an intrinsic network of TSH production and utilization between different types of BM cells, and they suggest that local TSH may be an important homeostatic regulator of hematopoiesis mediated by TNFα.

e-mail: John.R.Klein@uth.tmc.edu
Introduction

Although the participation of the neuroendocrine system in the regulation of immunity has been known for many years, most studies into that process have examined the involvement of glucocorticoid, steroid hormones, and reproductive hormones. Considerably less is known about how hormones of the hypothalamus-pituitary-thyroid axis affect the immune response. There is, however, evidence that thyroid stimulating hormone (TSH) can be produced by cells of the immune system, and that it is used by hematopoietic cells as seen from TSHR expression on hematopoietic cells, and by its capacity to influence various immunobiological activities in a TSH-dependent manner. Studies by Smith, et. al., demonstrated TSH secretion by human peripheral blood leukocytes stimulated with staphylococcus enterotoxin A\(^1\). Subsequent work revealed TSH gene expression in activated mouse spleen cells\(^2\), and secretion of TSH by mouse splenic mononuclear cells, in particular dendritic cells (DCs) and to a lesser extent T cells and B cells\(^3\). Human peripheral blood leukocytes have been shown to bind TSH in radiolabeled binding studies\(^4,5\) and by flow cytometry using biotin-labeled TSH\(^6\). Similar findings have been reported for murine splenic mononuclear cells\(^7\), collectively indicating that the TSH receptor (TSHR) is selectively expressed on cells of the peripheral immune system.

In mice TSH also been shown to influence developmental/immune regulatory functions of intestinal intraepithelial lymphocytes (IELs) involved in the recruitment and/or maturation of specific IEL subsets, most notably the CD8\(\alpha\)\(\beta\) TCR\(\alpha\)\(\beta\) IELs\(^8,9\). This appears to occur through a local TSH-mediated network between TSH-producing intestinal epithelial cells and TSHR\(^+\) IELs\(^10\). Within the bone marrow (BM), the expression of surface TSHR has been shown by immunoprecipitation\(^3\), though the types of cells of the BM that express the
TSHR was not determined, nor was the capacity of BM cells to produce TSH examined. Functionally, TSH has been shown to significantly influence BM cytokine synthesis, including tumor necrosis factor-α (TNFα)³.

Given the heterogeneity of BM cells and the basic role of the BM as a source of hematopoietic cells destined for secondary lymphoid tissue, it will be important to understand the cells involved in the production and use of TSH locally, and to characterize the biological significance of this system as a regulator of hematopoietic homeostasis. In the present study we have defined the subsets of BM cells, which produce and use TSH, and we provide new data linking TSH-induced synthesis of TNFα, an important cytokine with hematopoietic-regulating activities, to specific population(s) of BM cells.
Materials and methods

Mice
Female BALB/c mice, 6-10 weeks of age, were purchased from the National Cancer Institute (Frederick, MD) and were housed at the University of Texas Health Science Center Dental Branch.

Antibodies and reagents
Antibodies used in this study were: FITC-anti CD45 (leukocyte-common antigen) (30-F11); FITC-anti-CD11b (M1/70); FITC-anti-B220 (RA3-6B2); FITC-anti TCRβ (H57-597); biotin-labeled mouse IgM (anti-TNP) (G155-228); purified rat IgM (ER4-22); anti-CD16/32 Fc receptor block; streptavidin-phycoerythrin and streptavidin-cychrome (BD-PharMingen; San Diego, CA, all reagent); rabbit anti-mouse TSH (Accurate Chemicals; Westbury, NY); PE-anti-Ter-119 (Caltag, South San Francisco, CA); PE-anti Thy-1 (5a-8); biotin-anti rabbit antibody (Vector Laboratories; Burlingham, CA); biotin-labeled monoclonal anti-mouse TSHβ (1B11)11; anti-Fc receptor CD16 (2G2.4) tissue culture supernatant (American Type Culture Collection; Rockville, MD); and biotin-labeled human recombinant TSHβ (Sigma Chemicals; St. Louis, MO); human pituitary TSHαβ (98% pure iodination grade) (Calbiochem; San Diego, CA); E. coli lipopolysaccharide (Sigma). Biotinylation of mAb 1B11 and human recombinant TSHβ was done using published protocols from our laboratory11; purification of mAb 1B11 was done as previously described11.

Intracellular TSH staining and TSHR staining
Staining for the presence of intracellular TSHβ was done according to techniques developed for intracellular cytokine staining12. Briefly, 1.5x10⁶ cells were reacted for 10 min at 4°C with CD16/32 Fc receptor blocking reagent (BD-PharMingen). PE- or FITC-labeled anti-
CD11b, anti-CD45, anti-TCRβ, anti-B220, or anti-Thy-1 Mab was added for 20 min at 4°C. Cells were collected by centrifugation and suspended in 200 µl of cytofix/cytoperm (BD-PharMingen) for 20 min at 4°C, washed twice with perm-wash (BD-PharMingen), reacted for 20 min with 3 µg of rat IgM blocking antibody and washed with perm-wash. Cells were reacted for 20 min at 4°C with 50 µl of perm-wash containing 0.3 µg of biotinylated anti-TSHβ (mAb 1B11) or biotinylated mouse IgM for control staining. Cells were washed with perm-wash and reacted with streptavidin-PE or streptavidin-cychrome (BD-PharMingen) for 20 min at 4°C, washed, and fixed in 2% formalin. Staining for expression of surface TSHR was done by reacting 1 x 10^6 freshly-isolated BM cells with 8 µg of biotinylated recombinant TSHβ for 30 min at room temperature. Cells were washed and reacted with FITC-anti-CD11b or PE-anti-TER-119 plus streptavidin-PE or streptavidin-cychrome for 20 min at 4°C. Cells were washed and fixed in 2% formalin.

**Cell purification by magnetic-activated cell sorting (MACS)**

Purification of CD11b^+ and CD11b^- BM cells was done by positive and negative autoMACS cell separation (Miltenyi Biotec; Auburn, CA). Briefly, 15 x 10^6 freshly-isolated BM cells were reacted with 1 ml of anti-CD16 tissue culture supernatant for 10 min at 4°C. Cells were centrifuged and washed with labeling buffer (PBS, pH 7.2, supplemented with 2mM EDTA) and 35 µl of biotin-labeled anti-CD11b was added for 20 min at 4°C. Cells were washed with labeling buffer and 20 µl of streptavidin microbeads (Miltenyi) was added in 180 µl of labeling buffer for 15 min at 4°C. Cells were washed, suspended in 1 ml of separation buffer (PBS, pH 7.2, supplemented with 2 mM EDTA plus 0.5% BSA), and applied to autoMACS. Positive and negative cell populations were separated by autoMACS using the manufacturer’s protocols.
In vitro cell culture, TSH and TNFα immunoassays

For detection of secreted TSH, MACS-Purified CD11b^+ and CD11b^- BM cells were cultured for 18 hrs at a density of 1 x 10^6 cells/ml in RPMI-1640 containing 10% (v/v) FBS, 2 mM L-glutamine, 25 mM HEPES, 100 U/ml penicillin, and 100 µg/ml streptomycin (Sigma). TSH-induction of TNFα by TSH was done by culturing 1 x 10^6 MACS-sorted CD11B^+ and CD11b^- BM cells for 18 hrs in supplemented RPMI-1640 with graded doses of human TSH.

Cell-free BM supernatants were collected and coated overnight onto High-Binding type I EIA/RIA Strip Plates (Costar; Corning, NY). Wells were washed with PBS containing 0.05% Tween-20 (wash buffer), blocked for 1 hr at room temperature with blocking buffer (eBioscience; San Diego, CA), and washed x3 with wash buffer. Rabbit anti-mouse TSH antibody (1:200) was added for 1 hr at room temperature. Wells were washed and biotinylated anti-rabbit antibody (2µg/ml) was added for 1 hr at room temperature. Wells were washed x3 with wash buffer and 1:250 of streptavidin-horse radish peroxidase (eBioscience) was added for 30 min at room temperature. Wells were washed and O-phenylenediamine was added for 30 min at room temperature; colorimetric changes were measured at 490 nM using an automated ELISA reader (Molecular Devices, Sunnyvale, CA). Estimates of cell culture-derived TSH were determined from a standard curve of reactivity of anti-TSH antisera to serially-diluted (50–1.3 ng/ml) amounts of recombinant human TSHb.

TNFα secretion by BM cells was measured using a commercial assay (eBioscience) using the manufacturer’s protocols and reagents. Colorimetric changes were measured at 450 nM. Statistical analyses of data was determined by Student’s t-test for unpaired observations.

RT-PCR analyses
Procedures for RNA extraction and cDNA preparation have been previously reported\textsuperscript{3,9}.

Primer used were:

\begin{tabular}{ll}
TSHR forward & 5’-GACTCATCTGAAGACCATAACCAGTCTTGGCA-3’ \\
TSHR reverse & 5’-CATGTAAGGGTTGCTGATTTC-3’ \\
actin forward & 5’-ATGGATGACGATATCGCTG-3’ \\
actin reverse & 5’-ATGAGGTAGTCTGTCAGGT-3’ \\
\end{tabular}

Amplification conditions consisted of 50 cycles with 1 min at 95°C, 1 min at 50°C, and 1 min at 72°C for TSHR, and 30 cycles with 45 sec at 95°C, 45 sec at 50°C, and 30 sec at 72°C for \( \beta \)-actin.
Results

TSH production by bone marrow hematopoietic cells is linked to a subset of CD11b⁺ cells

Based on our previous studies demonstrating a role for TSH in the production of BM TNFα³, experiments were done to define the mechanisms through which TSH is responsible for regulating TNFα secretion. Thus, to identify population(s) of cells that might serve as a source of BM-derived TSH, BM cells were stained for intracellular TSH using procedures adapted from techniques employed for intracellular cytokine staining using a mAb to mouse TSHβ. Shown in Figure 1, BM cells, depending upon the cell population, expressed varying amounts of intracellular TSH in that approximately half of the lymphocyte precursor population of BM cells (Figure 1, R1) expressed low levels of intracellular TSH, three-fourths of the monocyte-macrophage precursor population (R2) and granulocyte precursor population (R3) expressed intracellular TSH at high levels.

To confirm that TSH-synthesizing cells in BM were hematopoietic cells and not

Figure 1. Intracellular TSH staining of bone marrow cells. Cells were stained with anti-mouse TSH mAb 1B11. BM populations are shown in the scattergram as lymphocyte precursors (R1), monocyte-macrophage precursors (R2), and granulocyte precursors (R3). Staining is compared to the reactivity of biotin-labeled mouse IgM antibody for each group. Data are representative of three experiments.

Figure 2. Characterization of TSH-producing bone marrow cells by intracellular TSH staining with anti-CD45 (leukocyte-common antigen) and anti-CD11b. Nearly all BM cells in regions R1, R2, and R3 (described in Figure 1 legend) that express intracellular TSH are hematopoietic cells (CD45⁺) and not stromal cells (CD45⁻), and are affiliated with a subset of CD11b⁺ cells. Data are representative of two experiments.
stromal cells, freshly-isolated cells were stained for intracellular expression of TSH in conjunction with anti-leukocyte-common antigen (CD45) mAb, and with anti-CD11b staining given the high reactivity of cells in the monocyte-macrophage and granulocyte lineages populations described above. Shown in Figure 2, nearly all BM intracellular TSH+ cells were CD45+, indicating that TSH production in the BM occurs from hematopoietic cells. Moreover, the majority of intracellular TSH+ cells in each group belonged to a population of CD11b+ cells, though a minor proportion (6%) of the total (82%) intracellular TSH+ cells in the monocytic precursor group were intracellular TSH+ cells (Figure 2, R2).

Three additional markers were used in conjunction with intracellular TSH staining. Anti-TCRβ was used to identify mature T cells, anti-B220 was used to identify developing B cells, and anti-Thy-1 was used as a marker of a subset of very early stem cells in the BM. Note that although a few TCRβ+ cells, B220+ cells, and Thy-1+ cells were intracellular TSH+, by far the largest number of TSH producing cells in the BM resided within the TCRβ−, B220−, Thy-1− populations (Figure 2).
3). These data coupled with those described in Figure 2 indicate that TSH synthesis within the BM is primarily affiliated with a population of CD11b⁺, TCRβ⁻, B220⁻, Thy-1⁻ BM cells.

To better define the relationship between BM cells and TSH secretion, and to confirm that intracellular TSH⁺ cells actively produce TSH, freshly-isolated BM cells were sorted by MACS into CD11b⁺ and CD11b⁻ groups and cells from each group were cultured overnight as described in Materials and methods. Cell-free supernatants were recovered and screened for TSH activity by ELISA. As shown in Figure 4, although both CD11b⁺ and CD11b⁻ BM cells secreted TSH, there were statistically-significant (p < 0.01) higher levels of TSH produced by CD11b⁺ cells (6.12 ± 0.38 ng/ml, N = 3) compared to CD11b⁻ cells (3.40 ± 0.18 ng/ml, N = 5). Previous studies from our laboratory reported murine TSH blood serum levels in the range of 20-40 ng/ml¹². Although it is difficult to equate TSH produced in vitro with that of TSH produced under normal physiological conditions in vivo, it is interesting that the concentration of TSH produced by CD11b⁺ BM cells in our assay was only slightly lower than that present in the circulation of normal mice. Collectively, these findings indicate that the CD11b⁺ cell population is the primary source of TSH in the murine BM.

The TSHR is primarily expressed on CD11b⁻ bone marrow cells
Expression of the TSHR gene was demonstrated by RT-PCR analyses using whole unfractionated BM cells (Figure 5A). To define the population(s) of BM cells that express TSHR, flow cytometric analysis was done using biotinylated recombinant TSHβ in two-color staining protocols with anti-CD11b staining. These experiments revealed a selective distribution of TSH-responsive cells in that the greatest number of TSHR\(^+\) cells in the BM were present in the lymphocyte precursor (R1) population of CD11b\(^-\) cells (Fig. 5B). Although some CD11b\(^+\) cells also were TSHR\(^+\), within regions that contained the greatest numbers of CD11b\(^+\) cells, i.e., the monocyte precursor (R2) and granulocyte precursor (R3) groups, most of those cells were TSHR\(^-\) (Fig. 5B). These patterns were confirmed in analyses of several BM preparations as shown in Table 1, which indicates that overall there were statistically more TSHR\(^+\) cells among CD11b\(^-\) cells than CD11b\(^+\) cells.

To determine whether TSHR\(^+\) cells consist of erythroid precursor population, BM cells were stained for expression of TSHR in conjunction with mAb Ter-119, a marker of murine erythroid precursors\(^16\). Shown in Figure 5B, within all three cell populations (R1, R2, and R3), the majority of the TSHR\(^+\) cells were located in the Ter-119\(^-\) cell population, thus confirming that erythroid precursors are not a primary TSH-responsive cell population of murine BM.

<table>
<thead>
<tr>
<th>Table 1. Expression of TSHR on bone marrow cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of cells within a group expressing TSHR</td>
</tr>
<tr>
<td>Lymphocyte precursors</td>
</tr>
<tr>
<td>CD11b(^-)</td>
</tr>
<tr>
<td>48.5±5.1(^{a,b,c})</td>
</tr>
</tbody>
</table>

\(^{a}\) p<0.01 compared to CD11b\(^+\) monocyte precursors and CD11b\(^-\) granulocyte precursors.  
\(^{b}\) p<0.001 compared to CD11b\(^-\) lymphocyte precursors and CD11b\(^-\) granulocyte precursors.  
\(^{c}\) p<0.05 compared to CD11b\(^+\) monocyte precursors.  
\(^{d}\) p<0.01 compared to CD11b\(^+\) lymphocyte precursors.  
\(^{e}\) p<0.025 compared to CD11b\(^-\) and CD11b\(^+\) granulocyte precursors.  
\(^{f}\) p>0.05 compared to CD11b\(^+\) monocyte precursors.  
Data are mean values ± SEM of four experiments. Statistical analyses were done using Student’s \(^t\)-test for unpaired observations.
The effect of TSH stimulation of BM cells was evaluated using CD11b⁺ and CD11b⁻ MACS-purified cells across a range of hormone concentrations. As seen in Figure 6, TSH had no significant effect on TNFα production by CD11b⁺ cells when tested across a 1,000-fold range of TSH concentrations. Lipopolysaccharide (LPS) at a concentration of 1,000 ng/ml (determined empirically to be the optimal stimulatory concentration [data not shown]) induced high levels of TNFα, demonstrating that although those cells did not produce TNFα when stimulated with TSH, they were capable of TNFα production. In contrast, to CD11b⁺ BM cells, CD11b⁻ cells, the cell population with the greatest proportion of TSHR⁺ cells, displayed a dose-dependent increase in levels of TNFα production following TSH stimulation, which was approximately half of that produced by LPS-stimulated cells (Figure 6). Although these findings are consistent with the observation that TSH stimulates the release of TNFα from TSHR⁺ CD11b⁻ BM cells, additional work will be needed to precisely define the network of hormone production and utilization among BM cells.

Figure 6. TSH-induced secretion of TNFα by CD11b⁺ and CD11b⁻ BM cells. MACS-sorted cells were cultured with graded doses of human TSH as described in Materials and methods. Note the significant (p<0.05) increase in TNFα secretion in TSH-stimulated vs. unstimulated CD11b⁻ cells, the predominant TSHR⁺ cells population (Figure 5). Among CD11b⁺ cells there was no significant difference in TNFα production compared to unstimulated cultures. TNFα production by LPS-stimulated cultures is shown for comparison. Data are mean values ± SEM of 2-5 samples per group.
Discussion

TNFα has been shown to have pleiotropic effects on hematopoiesis depending upon the target cells involved and/or the stage of development of those cells. Moreover, excessive levels of BM TNFα are believed to be linked to hematopoietic failure of allogeneic bone marrow transplantation\(^1\), and to be involved in the pathogenesis of aplastic anemia, particularly when combined with granulocyte-monocyte colony-stimulating factor (GM-CSF)\(^2\). Developmentally, TNFα has been reported to have stimulatory effects in IL-3- and GM-CSF-supplemented cultures of human CD34\(^+\) hematopoietic progenitor cells (HPCs) during shortterm culture\(^3\), and to have inhibitory effects on longterm HPC cell cultures\(^4\). Other studies indicate that the inhibitory activity of TNFα is mediated through the p55 TNFR and that this occurs primarily for the more mature HPC populations, whereas TNFα-mediated inhibitory effects are associated with the p75 TNFR on early HPCs\(^5,6\). While these studies provide evidence for a role for TNFα in the regulation of hematopoiesis, they do not determine how TNFα activity is controlled locally within the BM. The finding reported here that TSH stimulation of CD11b\(^-\) BM cells results in increased levels of TNFα production (Fig. 6) suggests a role for TSH in that process. It is important to note, however, that the effect of TSH on TNFα synthesis need not be direct even though CD11b\(^-\) cells express TSHR. In fact, a proportion of both CD11b\(^+\) and CD11b\(^-\) cells are TSHR\(^+\) (Fig. 5 and Table 1), and the poteniating effect of TSH on TNFα activity could occur through the elaboration of TNFα-regulating intermediates produced by TSHR\(^+\) cells.

In conjunction with GM-CSF, TNFα has been shown to play an important role in the generation of DCs from precursors in the BM. This has been demonstrated with regard to BM-derived Langerhans cells\(^7\), and for the development of DCs from early progenitor-stage...
Moreover, that effect may be the consequence of TNFα-mediated upregulation of GM-CSF receptors on DC precursors. Other studies have demonstrated that TNFα plus IL-3 in the absence of GM-CSF can mediate DC development from human cord blood, implying that TNFα can act synergistically with several growth-promoting factors, or possibly that it induces the secretion of other growth-promoting factors, but nonetheless further defining an important role for TNFα in the process of DC development within primary hematopoietic tissues. Additionally, a recent study of mouse BM-derived DC gene expression using DNA microarray assays demonstrated upregulation of TNFα genes in mature DCs relative to immature DCs. In that study, BM-derived DCs were generated by in vitro culture for 7 days with GM-CSF and IL-4; DC maturation was achieved by LPS stimulation for an additional 24 hrs. As yet, we have not examined TSH production of bone marrow cells after in vitro maturation, though we recently demonstrated that TSH is produced by purified splenic DCs. However, the relationship of those cells to the BM cells described here has not been determined, though these studies are currently under way.

Interestingly, significantly more CD11b+ BM cells, depending upon the population, expressed intracellular TSH than CD11b− cells, yet there was only a two-fold difference in the amount of secreted TSH from the former versus the latter cells. Although the basis for that difference in intracellular TSH production by those two populations is not yet evident, the presence of intracellular TSH in CD11b+ BM cells would imply that TSH is more rapidly available from those cells compared to CD11b− cells. It should be pointed out that our studies to date have examined TSH secretion without exogenous stimulation. Experiments are currently underway to determine kinetic TSH release from CD11b+ and CD11b− cells after culture with stimuli with mitogenic, cell-differentiating, and/or growth-promoting potential.
In summary, despite progress in understanding how hormones, neuroendocrines, and neuropeptides collaborate in the regulation of immunity, many basic aspects of those interactions have yet to be delineated. The findings reported here suggest that locally synthesized TSH may be involved in that process. Because hormones such as TSH are present in the blood and thus have the potential to reach a vast number of different organs and tissues, TSH-mediated effects operating across classical endocrine pathways would be difficult to regulate from an immunological perspective, whereas the local manufacture and release of TSH disseminated across short distances in a manner analogous to the elaboration and use of cytokines and chemokines might logistically resolve this problem.
References


18. Martinez-Jaramillo G, Lores-Figueroa E, Gomez-Morales E, Sanchez-Valle E, Mayani H. Tumor necrosis factor-α levels in long-term marrow cultures from patients with aplastic...
2001;68;144-148.

19. Caux C, Leland S, Favre C, Duvert V, Mannoni P, Banchereau J. Tumor necrosis factor-
alpha strongly potentiates interleukin-3 and granulocyte-macrophage colony-stimulating
1990;75:2292-2298.

of early hematopoiesis by tumor necrosis factor alpha is followed by inhibition of

Bifunctional effects of tumor necrosis factor alpha (TNFα) on the growth of mature and
primitive human hematopoietic progenitor cells: involvement of p55 and p75 TNF

22. Jacobsen FW, Rothe M, Rusten L, et. al., Role of the 75-kDa tumor necrosis factor
receptor: Inhibition of early hematopoiesis. Proc Natl Acad Sci USA. 1994;91:10695-
10699.

23. Caux D, Dezutter-Dambuyant C, Schmitt D, Banchereau J. GM-CSF and TNF-α

24. Caux C, Dezutter-Dambuyant C, Schmitt D, Banchereau J. Tumor necrosis factor-α
cooperates with interleukin 3 in the recruitment of a primitive subset of human CD34+


Figure Legends

Figure 1. Intracellular TSH staining of bone marrow cells. Cells were stained with anti-mouse TSHβ mAb 1B11. BM populations are shown in the scattergram as lymphocyte precursors (R1), monocyte-macrophage precursors (R2), and granulocyte precursors (R3). Staining is compared to the reactivity of biotin-labeled mouse IgM antibody for each group. Data are representative of three experiments.

Figure 2. Characterization of TSH-producing bone marrow cells by intracellular TSH staining with anti-CD45 (leukocyte-common antigen) and anti-CD11b. Nearly all BM cells in regions R1, R2, and R3 (described in Figure 1 legend) that express intracellular TSH are hematopoietic cells (CD45+) and not stromal cells (CD45−), and are affiliated with a subset of CD11b+ cells. Data are representative of two experiments.

Figure 3. TSH-producing bone marrow cells are not associated with mature T cells, developing B cells, or Thy-1+ bone marrow cells. To further characterize the cellular source of TSH within the BM, cells were stained with anti-TCRβ chain mAb as an indicator of mature αβ T cells, with anti-B220 mAb as an indicator of developing B cells, or with anti-Thy-1 mAb since low levels of Thy-1 are expressed on a population of early bone marrow stem cells. Note that in all cell populations (R1, R2, and R3), only a very small percentage of the total intracellular TSH+ cells were mature T cells, developing B cells, or stem cells, further confirming that the primary source of TSH in the bone marrow is a population of CD11b+ cells.
Figure 4. Secretion of TSH by MACS-sorted CD11b+ and CD11b− bone marrow cell. Cells were cultured as described in Materials and methods. Consistent with the findings for intracellular TSH staining (Figure 2), there was a significant increase (p<0.01) in TSH produced by CD11b+ BM cells than CD11b− cells, indicating that CD11b+ cells are a major source of TSH in the bone marrow. Data are mean values ± SEM of 3 or 5 samples per group.

Figure 5. Evidence for TSHR expression in murine bone marrow. (A) RT-PCR analyses of TSHR in whole BM cells indicates active expression of the TSHR gene. (B) Two-color flow cytometric analyses of BM cells in regions R1, R2, and R3 (see Figure 1 legend) stained with biotinylated recombinant TSHβ and for identification of TSHR+ cells and anti-CD11b indicates a preponderance of TSHR+ cells among the lymphocyte precursor population; the overall lack of TSHR staining for Ter-119 cells indicates that cells in the region R1 are not erythrocyte precursors. Data are representative of 2-4 experiments.

Figure 6. TSH-induced secretion of TNFα by CD11b+ and CD11b− BM cells. MACS-sorted cells were cultured with graded doses of human TSH as described in Materials and methods. Note the significant (p<0.05) increase in TNFα secretion in TSH-stimulated vs. unstimulated CD11b− cells, the predominant TSHR+ cells population (Figure 5). Among CD11b+ cells there was no significant difference in TNFα secretion of TSH-stimulated cells compared to unstimulated cells. TNFα production by LPS-stimulated cultures is shown for comparison. Data are mean values ± SEM of 2-5 samples per group.
An intrinsic thyrotropin-mediated pathway of TNFα production by bone marrow cells

Heuy-Ching Wang, Jolene Dragoo, Qin Zhou and John R Klein